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# The Protective Effect of Indole Alkaloid Vincanine Against Hypoxia-Induced Vasorelaxation Model of Rat Aorta

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**Using conventional organ bath procedures, the current study sought to determine how vincanine hydrochloride affected vasorelaxation brought on by hypoxia in rat aortic rings. To induce hypoxia, we used a glucose-free Krebs solution that was infused with 95% N2 and 5% CO2. After 60 minutes of hypoxia, the effect of vincanine was evaluated on aortic rings that were precontracted with either 50 mM KCl or 1 $\mu$M phenylephrine (PE). The effect of vincanine was more noticeable in aortic rings that had been precontracted by PE as opposed to KCl. Additionally, when verapamil, a blocker of L-type VDCCs, was preincubated with endothelium-intact aortic rings and KCI was used for precontraction, the effect of vincanine on hypoxia-induced vasorelaxation was significantly reduced. Vincanine inhibited hypoxia-induced vasorelaxation in aortic rings precontracted with PE in a calcium-free buffer. Furthermore, the presence of glibenclamide, a specific inhibitor of ATP-sensitive K+-channels (KATP), and tetraethylammonium chloride (TEA), a nonspecific inhibitor of calcium-activated large conductance K+-channels (BKca), significantly reduced the effect of vincanine on hypoxia-induced vasorelaxation. The removal of the endothelium also had a significant impact on the effect of vincanine on hypoxia-induced vasorelaxation. The present findings showed that alkaloid vincanine isolated from the leaves of Vinca minor H. significantly abolished the hypoxia-induced vasorelaxation in rat aorta. The obtained results suggest that vincanine may protect the rat aorta against hypoxic injuries in the vasculature.**

**Keywords:** Hypoxia; Rat Aorta; Vasorelaxation; Vincanine.

The world's top cause of death is still cardiovascular diseases (CVD), with hypertension being the main factor contributing to this high death rate.[1] Approximately one-third of annual worldwide deaths can be attributed to CVD[2]. Moreover, elevated blood pressure, or hypertension, is closely associated with at least half or more of cases involving ischemic stroke, hemorrhagic stroke, ischemic heart disease, cardiomyopathy, aortic aneurysms, and peripheral vascular disease[3]. Unfortunately, this burden is increasing, despite the advancements in therapeutic treatments, particularly for elderly individuals[4,5,6].

Accordingly, one of the most pressing issues facing contemporary medicine is the creation of fresh strategies for the effective treatment of

arterial hypertension that are based on the most recent developments in molecular pharmacology. In addition, knowledge regarding the pharmacological characteristics of blood vessel-specific targets is especially crucial as they have a direct bearing on the aetiology of arterial hypertension. A specific focus is on clarifying the mechanisms governing the modulation of smooth muscle $Ca^{2+}$ transporting systems, as their failure results in the emergence of pathogenic processes inside blood vessels. Disturbances in the contractile activity of SMC-which is controlled by the intracellular $Ca^{2+}$ ion level upheld by the sarcoplasmic reticulum (SR) and plasmalemma-play a pivotal role in this instance(Jackson, 2000). It is well known that abnormalities in the contractile activity of SMCs associated with arterial hypertension are directly linked to their $Ca^{2+}$ transport system malfunction[7]. Simultaneously, particular focus is placed on naturally occurring biologically active chemicals derived from plants. These compounds exhibit a broad spectrum of pharmacological effects and specifically interact with different kinds of ion channels.

The study focused on Vinca erecta Regel & Schmalh, a plant primarily found in Central Asia's mountainous region of Uzbekistan. Through analyzing the roots and aerial parts of Vinca erecta, it was discovered that the plant contains a high concentration of indole alkaloids. These specific alkaloids, known as Vinca erecta, have gained significant recognition in the medical field for their effectiveness in treating cancer, malaria, and cardiac arrhythmia[8]. Researchers have recently explored the modification of these alkaloids to generate new bioactive compounds. One particularly promising compound is norfluorocurarine (vincanine), which was extracted from Vinca erecta. By synthesizing new quaternary halide derivatives of vincanine, researchers have expanded the scope of organic synthesis possibilities. Additionally, it is important to note that the quaternary base of norfluorocurarine, fluorocurarine, and its natural derivatives exhibit zwitterionic properties of various natures[9]. Recently we found that vincanine effectively protects rat aorta against hypoxia- induced vasorelaxation. In the present study, we aim to explore the mechanism by which vincanine protects rat aorta against hypoxia-induced vasorelaxation. This should help to gain novel insight into the mechanisms involved in the cardioprotective effect of vincanine[10].

## MATERIALS AND METHODS

### Chemicals

All chemicals were of analytical grade commercially available. Phenylephrine, L-NAME, methylene blue, indomethacin, glibenclamide, TEA and, $BaCl_2$ were obtained from Sigma Ltd Co., (St. Louis, MO, USA). Vincanine hydrochloride has been isolated as a Vincanine from the roots of Vinca erecta (fam. Apocynaceae) at the Institute of the Chemistry of Plant Substances of Academy of Sciences of Uzbekistan and was kindly provided by Shahobiddin Adizov**.**

### Tissue Preparation

Our institution's animal use committee authorized all experimental procedures and preoperative care guidelines. After being given sodium pentobarbital anesthesia, adult male Wistar rats weighing 200–250 g had their thorax opened, the thoracic aorta was swiftly removed, and the rats were then placed in Krebs solution, which contained the following concentrations of salt (in mmol/l): 118 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 2 mM $CaCl_2$, 1.2 mM $KH_2PO_4$, and 11 mM glucose. The endothelium was handled extremely carefully during the entire dissection process to prevent accidental injury. After being cleared of fat and connective tissue, the aorta was divided into rings that measured 2-3 mm in length[11].

### Aortic-ring contraction studies

Two stainless hooks were used to mount the aortic rings; one was fastened to the organ bath's bottom (Radnoti Glass, NSW, AUS), and the other was attached to a force transducer. Krebs solution was superfused into the organ bath, which was kept at 37 oC and bubbled with a gas mixture consisting of 95% $O_2$ and 5% $\tilde{N}\hat{I}_2$. For 60 minutes, the Krebs solution was changed at least twice while the aortic rings were equilibrated in the solution with a tension of 1 g. A force transducer (FT-03; Grass Instrument Company, USA) attached to a computer-based data acquisition system (PowerLab, ADInstruments) connected to a chart recorder Endim 621-02 (Germany) was used to record the aortic ring contraction isometrically.

**Experimental Protocols**

The effect of vincamine on hypoxia-induced vasorelaxation was assessed using aortic rings incubated in a glucose-free Krebs solution with a constant supply of 95% $N_2$ / 5% $CO_2$. After a 60-minute period of hypoxia which is commonly used in these studies, aortic rings were precontracted with 50 mM KCl or 1µM phenylephrine (PE). The reduction in force upon hypoxia was described as a percentage of the contraction force caused by 50 mM KCl or 1µM PE just before aeration with 95% $N_2$ / 5% $CO_2$. This maximum hypoxic vasorelaxation occurred at around 60 minutes.

To examine the involvement of the voltage-gated $Ca^{2+}$-channels (VGCCs) in the effect of vincanine on hypoxia-induced vasorelaxation its effects in the presence of verapamil (0.1-1 µM), an L-type $Ca^{2+}$- channel inhibitor, were studied.

The effects of vincanine on hypoxia-induced vasorelaxation were examined in relation to its role in $Ca^{2+}$ release from the sarcoplasmic reticulum (SR). Specifically, the effects were examined in relation to PE-induced contraction of endothelium-denuded aortic rings in $Ca^{2+}$-free buffer containing EGTA (1 mM). In the second series of experiments, the effects of vincanine on hypoxia-induced vasorelaxation and its effects on caffeine-induced contraction were investigated in order to investigate the role of $Ca^{2+}$ release from the SR in these processes.

Tetraethylammonium chloride (TEA), a nonspecific inhibitor of the calcium-activated large conductance $K^+$ channel ($BK_{Ca}$), glibenclamide, a specific inhibitor of the ATP-sensitive $K^+$ channel ($K_{ATP}$), and $BaCl_2$, a specific inhibitor of the inward rectifying $K^+$ channels ($K_{IR}$), were studied in conjunction with vincanine to assess the contribution of the $K^+$-channels to the effect of the drug on hypoxia-induced vasorelaxation. Since the action of medicines on $K^+$-channels is more effective in endothelium-intact aortic rings, these investigations were conducted on these rings after they had been precontracted with 30 mM KCl to depolarize the membrane and activate $K^+$-channels[12].

After removing the endothelium from ring specimens by rubbing the intimal surface with a cotton ball, the absence of ACh-induced relaxation was considered as evidence of successful denudation. This allowed researchers to evaluate the involvement of the endothelium in the effect of vincanine on hypoxia-induced vasorelaxation. The effects of PE-induced contraction of endothelium-intact aortic rings preincubated with L-NAME (nitro-L-arginine methyl ester, an inhibitor of NO synthase), methylene blue (a guanylyl cyclase inhibitor), and indomethacin (a cyclooxygenase inhibitor) were investigated in order to better understand the role of the endothelium in the effect of vincanine on hypoxia-induced vasorelaxation.

**Statistics**

This article uses the mean ± standard error of the mean (s.e.m.) of n observations to represent all data. To conduct statistical analysis, an unpaired Student's t-test was employed. The concentration-response curve was used to get the $EC_{50}$ and $IC_{50}$ values, or the drug concentrations that cause a 50% contraction or relaxation of the maximal response (EMax). The sigmoidal curve fitting method in Origin 7.0 (Microcal, Northampton, MA, U.S.A.) was used to generate these values. When $P < 0.05$ was reached, the differences between the experimental and control values were deemed significant.

## RESULTS AND DISCUSSION

**Impact of vincanine on vasorelaxation produced by hypoxia**

In control experiments, exposure of endothelium-intact aortic rings to Krebs solution gassed with 95% $N_2$ / 5% $CO_2$ for 60 minutes significantly reduced contractile response to KCl or PE. As shown in Fig. 1, the response of aortic rings to KCl (50 mM) and PE (1µM) was decreased by 55.3±4.1% and 51.2±4.4%, respectively, of the control response in normoxia. These results show that stimulated hypoxia-induced vasorelaxation which was more potent in aortic rings precontracted with PE than KCl indicating that the contractile response to PE is more sensitive to hypoxia than is that to KCl. It was found that the pretreatment of the endothelium-intact aortic rings with vincanine significantly reduced the vasorelaxation induced by hypoxia in aortic rings precontracted both with KCl and PE Fig.1. As demonstrated in Fig. 1, A the vincanine maximally inhibited hypoxia-induced vasorelaxation in the aortic rings precontracted with KCl (50 mM) from 44.7±4.1% to 17.4±3.7%

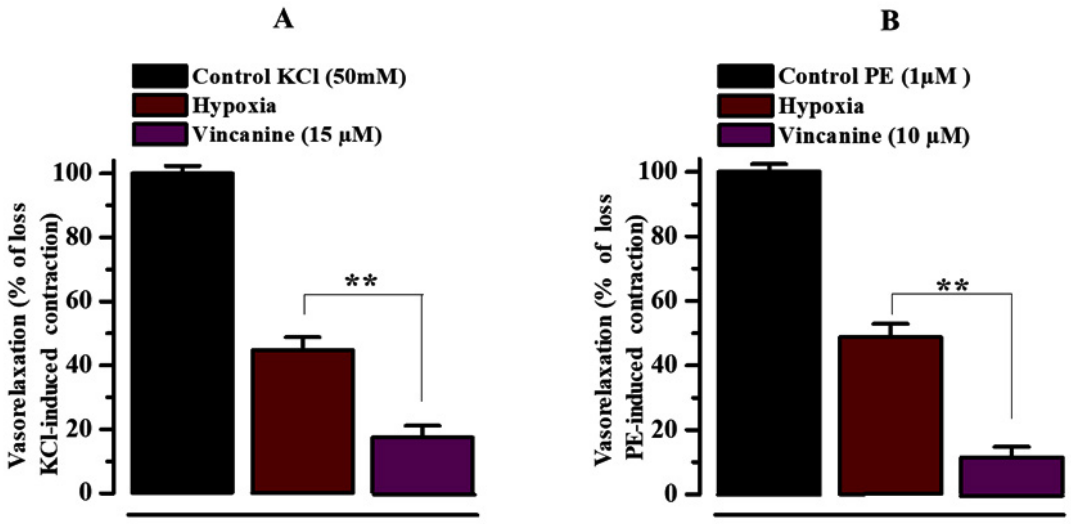


**Fig. 1.** Effect of vincaine on endothelium-induced vasorelaxation generated by hypoxia: intact rat aortic rings precontracted with phenylephrine and KCl (A) (B). Preincubated in Krebs solution gassed with 95 $N_2$ / 5% $CO_2$, the endothelium-intact aortic rings were precontracted with 50 mM KCl and 1µM phenylephrine (PE) for 60 minutes. The percentage of inhibition of the maximum contraction generated by PE and KCl was used to express vasorelaxation. The data (n = 6) are shown as mean ± SEM. **P<0.01, *P<0.05, compared to control

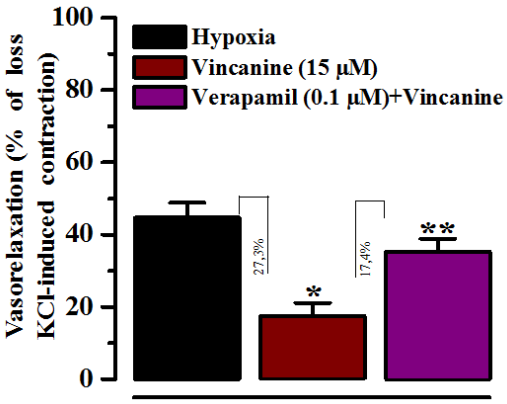


**Fig. 2.** Influence of verapamil on the hypoxia-induced vasorelaxation in endothelium in intact rat aortic rings precontracted with KCl was observed. Preincubated in Krebs solution gassed with 95 $N_2$ / 5% $CO_2$, the endothelium-intact aortic rings were precontracted with 50 mM KCl for 60 minutes. The percentage of inhibition of the maximum contraction induced by KCl was used to express vasorelaxation. The data (n= 6) are shown as mean ± SEM. * 0.05, ** 0.01, compared to control

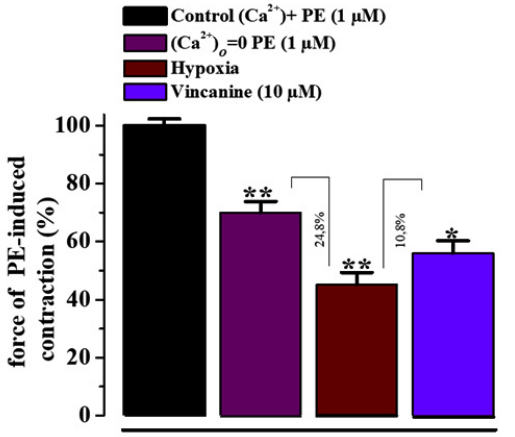


**Fig. 3.** Effect of vincanine on endothelium-induced vasorelaxation in $Ca^{2+}$-free Krebs solution with intact rat aortic rings precontracted by phenylephrine. The endothelium-undamaged aortic rings were precontracted with 1µM phenylephrine (PE) and preincubated for 60 minutes in a $Ca^{2+}$-free Krebs solution that was gassed with 95 $N_2$/5% $CO_2$. The percentage of inhibition of the maximum contraction generated by PE in the $Ca^{2+}$-free Krebs solution was used to indicate vasorelaxation. The information is displayed as mean ± SEM (n=4) * P<0.05, ** P<0.01, relative to the control

at the concentration of 15 μM. Similarly, on the aortic rings precontracted with PE (1 μM) vincanine maximally inhibited hypoxia-induced vasorelaxation from 48.8±4.4% to 11.5±3.2% at the concentration of 10 μM Fig. 1, B.

These results show that the effect of vincanine on the vasorelaxation induced by hypoxia is more potent in aortic rings precontracted with PE than with KCl. It was reported that vasorelaxation induced by hypoxia is mainly due to a reduction $[Ca^{2+}]_i$ in the smooth muscle cells resulting from the inhibition of $Ca^{2+}$ influx through VDCCs and calcium release from the SR via $IP_3Rs$[13,14]. To examine the involvement of the VDCCs in the inhibitory effect of vincanine on hypoxia-induced vasorelaxation its effects in the presence of verapamil, an inhibitor of L-type $Ca^{2+}$ channels, were studied. In these studies, was found that in aortic rings preincubated with verapamil (0.1 μM) and precontracted with KCl the inhibitory effect of vincanine on the vasorelaxation induced by hypoxia reduced from 27.3±3.7% to 17.4±3.7% Fig. 2. This result suggests that L-type VDCCs may be involved in the inhibitory effect of vincanine on hypoxia-induced vasorelaxation.

Vincanine also inhibited the hypoxia-induced vasorelaxation in the aortic rings precontracted with PE in the $Ca^{2+}$-free buffer. In these experimental conditions, PE-induced aortic ring contraction is mainly due to the release of $Ca^{2+}$ from SR through inositol 1,4,5-trisphosphate receptors ($IP_3Rs$)[15]. Findings displayed in Fig. 3 demonstrate the impact of vincanine on the vasorelaxation induced by hypoxia in these conditions reduced from 24.8±4.1% to 10.8±4.2%. These results indicate that the modulation of calcium release from the SR also may be associated with the inhibitory effect of vincanine on the vasorelaxation induced by hypoxia.

This suggestion was confirmed in experiments that examined the effect of vincanine on caffeine-induced contraction in a $Ca^{2+}$-free buffer which is mediated by $Ca^{2+}$ released from the SR by the mechanism of $Ca^{2+}$-induced $Ca^{2+}$-release (CICR)[16,17,18]. According to these studies, vincanine decreased the vasorelaxation brought on by hypoxia in intact aortic rings precontracted with caffeine (10 mM) in the $Ca^{2+}$-free buffer from 12.8±3.2% to 7.8±4.3%. Fig. 4. All of these findings point to the possibility that the modulation

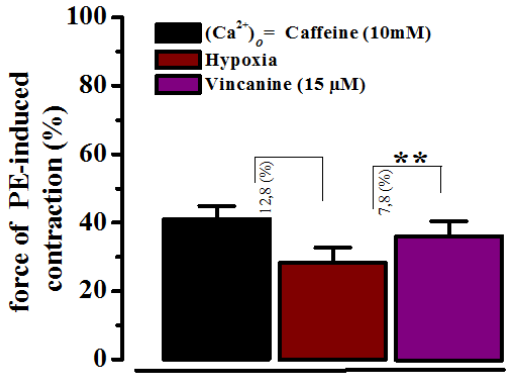


**Fig. 4.** Effect of the vincanine on the hypoxia - induced vasorelaxation in endothelium - intact rat aortic rings precontracted with caffeine in $Ca^{2+}$-free Krebs solution. Endothelium-intact aortic rings were preincubated for 60 min in $Ca^{2+}$-free Krebs solution gassed with 95 $N_2$ / 5% $CO_2$ and precontracted with caffeine (10mM). The percentage of inhibition of the maximum contraction induced by KCl was used to express vasorelaxation. The data (n=5) are shown as mean±SEM. **P<0.01, *P<0.05, compared to control

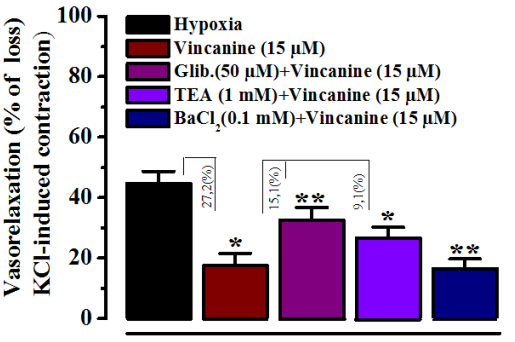


**Fig. 5.** Effect of $K^+$ channel blockers on hypoxia-induced vasorelaxation in intact aortic rings when vincanine is present. Aortic rings that were still in their whole underwent a 60-minute preincubation period in Krebs solution that was gassed with 95 $N_2$ and 5% $CO_2$, along with 10 ìM glibenclamide (Glib), 1 mM TEA, and 30 ìM $BaCl_2$. The rings were then precontracted with 30 mM KCl. The percentage of inhibition of the maximum contraction induced by KCl was used to express vasorelaxation. The data (n=4) are shown as mean ± SEM. **P<0.01, *P<0.05, compared to control

of L-type VDCC and the $Ca^{2+}$-induced $Ca^{2+}$-release pathway may be the mechanism by which vincanine inhibits hypoxia-induced vasorelaxation.

**The involvement of the $K^+$ - channels in the inhibitory effect of vincanine on hypoxia-induced vasorelaxation**

Numerous studies have demonstrated that the mechanism underlying vasorelaxant response to hypoxia may involve the activation of various types of $K^+$-channels, resulting in vascular hyperpolarization and relaxation. In particular, it was reported that the inward rectifier $K^+$-channels ($K_{IR}$ 2.1) which modulate basal tone in small-diameter coronary and cerebral arteries participated in their hypoxic vasorelaxation[19]. Also was shown that voltage-gated $K^+$-channels ($K_V$ 7) which control the tone of pulmonary and cerebral arteries may also be involved in hypoxic vasorelaxation[20].

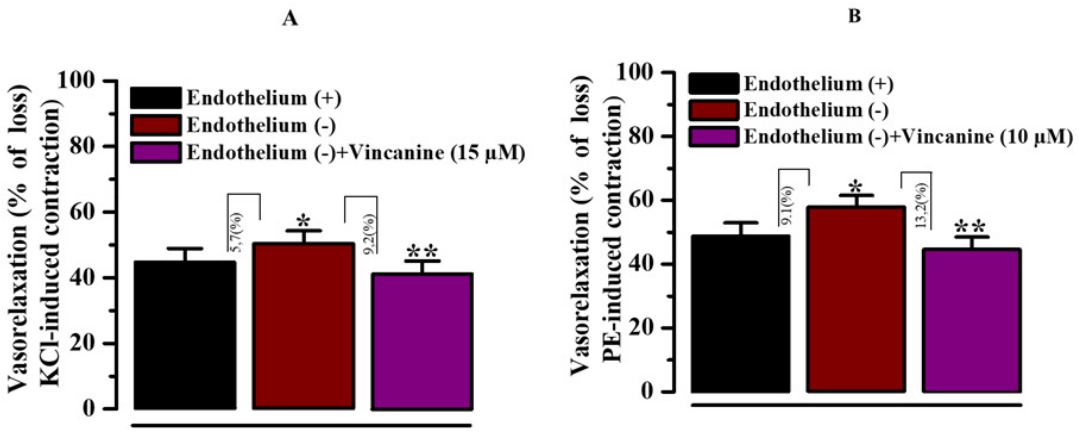


**Fig. 6.** Effect of vincanine on hypoxia-induced vasorelaxation in the endothelium - aortic rings precontracted with KCl (A) and phenylephrine (B), both intact and endothelium denuded. Preincubated in Krebs solution gassed with 95% $N_2$ / 5% $CO_2$, the aortic rings with intact (E+) and removed (E-) endothelium were precontracted with 50 mM KCl and 1µM phenylephrine (PE) for 60 minutes. The percentage of inhibition of the maximum contraction generated by PE and KCl was used to express vasorelaxation. The data (n=5) are shown as mean ±SEM. **P<0.01, *P<0.05, compared to control.

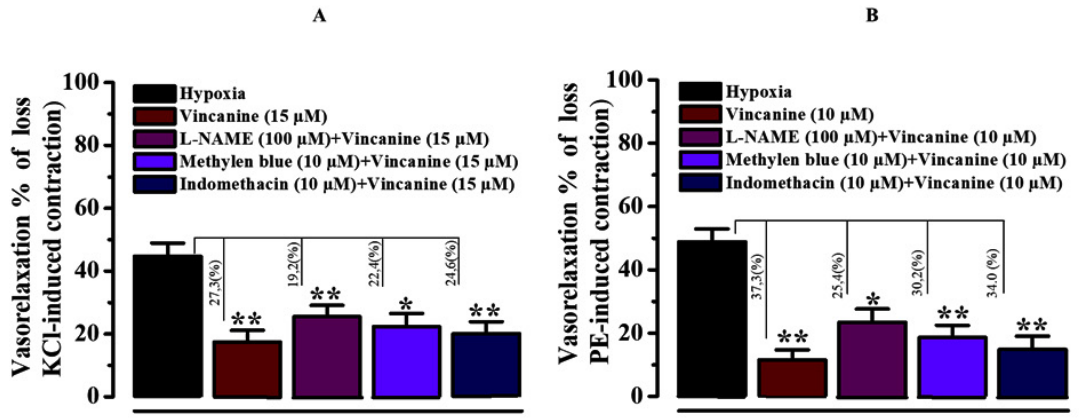


**Fig. 7.** Effect of vincanine on hypoxia - induced vasorelaxation in the endothelium - intact aortic rings preincubated with L-NAME, methylene blue, and indomethacin and precontracted with KCl (A) and phenylephrine (B). The aortic rings with intact (E+) endothelium - were preincubated for 60 min in Krebs solution gassed with 95% $N_2$ / 5% $CO_2$ in the presence of L-NAME (100µM), methylene blue (10µM) and indomethacine (10µM) and precontracted with 50 mM KCl and 1µM phenylephrine (PE). The percentage of inhibition of the maximum contraction generated by PE and KCl was used to express vasorelaxation. The data (n = 5) are shown as mean ± SEM. **P<0.01, *P<0.05, compared to control

Furthermore, it has been proposed that hypoxia may partially cause vasorelaxation by directly activating ATP-sensitive $K^+$-channels ($K_{ATP}$)[21]. $K_{ATP}$-channels are essential for vasodilation in response to metabolic demand and for maintaining resting blood flow in a number of vascular beds, most notably the coronary circulation[22].

Thus, we investigate in this work if various $K^+$-channel types are involved in the (inhibitory) action of vincamine on hypoxia-induced vasorelaxation. For this purpose, the inhibitory effect of vincamine on hypoxia-induced vasorelaxation was studied in the presence of specific inhibitors of $K_{ATP}$, $BK_{Ca}$ and $K_{IR}$-channels, glibenclamide (GLI), TEA, and $BaCl_2$, respectively. These studies were performed in the aortic rings precontracted with 30 mM KCl since in this condition effect of drugs on $K^+$ channels is more potent[23]. As shown in Fig. 5 in the intact aortic ring preincubated with glibenclamide (50 ìM) and precontracted with KCl (30 mM) the inhibitory effect of vincanine on hypoxia-induced vasorelaxation reduced from 27.2±4.1% to 15.1±4.2%. Similarly, in the presence of TEA (10 mM) the inhibitory effect of vincanine on hypoxia-induced vasorelaxation was reduced from 26.6±3.6% to 9.1±3.6%. By contrast, in the presence of $BaCl_2$ (100 ìM), the effect of vincanine on hypoxia-induced vasorelaxation reduced not significantly (Fig. 5). These results demonstrate that preincubation of aortic rings with glibenclamide significantly abolished the effect of vincanine on hypoxia-induced vasorelaxation suggesting that $K_{ATP}$ - channels could be involved in this effect.

**The involvement of the endothelium in the inhibitory effect of vincanine on hypoxia-induced vasorelaxation**

It was shown that a critical role in hypoxia-induced vasorelaxation plays changes in the mechanisms of endothelium-dependent contraction and relaxation mediated by several vasoactive factors[24,25]. Vasoconstrictors like thromboxane ($TXA_2$) and endothelin-1 (ET-1)[26], or vasodilators like nitric oxide (NO), prostacyclin ($PGI_2$), and endothelium-derived hyperpolarizing factor (EDHF), are the most significant among them. Thus, the aortic rings with endothelium removed were used to assess the impact of vincanine on hypoxia-induced vasorelaxation in order to examine the participation of endothelium. The vasorelaxation caused by hypoxia in aortic rings precontracted with KCl (50 mM) and PE (1 μM) increased by 5.7±2.4% and 9.1±3.7%, respectively, when the endothelium was removed by rubbing the intimal surface with a cotton ball (Fig. 6). These findings demonstrate that in aortic rings precontracted by PE, the ablation of the endothelium more dramatically increases the vasorelaxation brought on by hypoxia.

In similar experiments in the endothelium-denuded aortic rings precontracted with KCl (50 mM) and PE (1 μM) the effect of vincanine on hypoxia-induced vsorelaxation reduced from 27.2±3.7% to 9.2±3.6% and from 37.3±3.2% to 13.2±3.9%, respectively Fig. 6. These results indicate that removal of endothelium significantly reduced the effect of vincanine on hypoxia-induced vasorelaxation suggesting that this effect of vincanine is endothelium-dependent.

The effect of vincanine on endothelium-intact aortic rings preincubated with NOS inhibitors (L-NAME), guanylate cyclase-methylene blue, and cyclooxygenase-indomethacin was investigated in order to shed more light on the endothelium's role in hypoxia-induced vasorelaxation. As shown in Fig. 7,A in the intact aortic ring preincubated with L-NAME (100 μM) and methylene blue (10 μM) and precontracted with KCl (50 mM) the inhibitory effect of vincanine on hypoxia-induced vasorelaxation reduced from 27.3±3.7% to 19.2±3.6% and from 27.3±3.7% to 22.4±4.2%, respectively. At these time in intact aortic ring preincubated with L-NAME (100 μM) and methylene blue (10 μM) and precontracted with PE (1 μM) the inhibitory effect of vincanine on hypoxia-induced vasorelaxation was reduced from 37.3±3.2% to 25.4±4.1% and from 37.3±3.2% to 30.2±3.9% a respectively Fig. 7, B. In contrast preincubation of the intact aortic ring with indomethacine, a cyclooxygenase inhibitor, not significantly inhibited the effect of vincanine on hypoxia-induced vasorelaxation Fig.7. These results demonstrate that L-NAME and methylene blue significantly reduced the effect of vincamine on hypoxia-induced vasorelaxation suggesting that this effect of vincanine is endothelium-dependent and likely is associated with modulation of NO/sGC/cGMP/PKG pathway.

## CONCLUSION

According to the current research, the hypoxia-induced vasorelaxation in the rat aorta was greatly reduced by the alkaloid vincanine that was extracted from Vinca minor H. leaves. Vincanine may shield the rat aorta from hypoxic damage to the vasculature, according to the acquired results. This endothelium-dependent protective effect of vincanine is probably achieved by suppression of L-type VDCC and $Ca^{2+}$ release from SR in addition to modifying the NO/sGC/cGMP/PKG pathway. New therapeutic approaches for the prevention and treatment of a variety of cardiovascular disorders linked to ischemia/hypoxia may result from further research into the processes behind vincanine's protective action.

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**Conflict of interest**

The authors have declared that no conflict of interest exists.

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**Ethics approval**

The experimental protocols complied with the standards and requirements for the humane treatment of animals and the provisions of the Ethical Commission of the IBB at the National University of Uzbekistan. (Protocol No. 7 of 04/07/2022) on the use of laboratory animals. Preparations of isolated aortic segments were obtained using a known method.

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